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PRODUCT DATASHEET

Ready-to-Assay™ ChemR23 Chemoattractant Receptor Frozen Cells

CATALOG NUMBER: HTS071RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

ChemR23 was discovered as an orphan receptor related to the chemoattractant receptors C3a, C5a and FPR1, and expressed on dendritic cells and macrophages (Samson *et al.*, 1998). A ligand for ChemR23 was characterized as chemerin, a 15 kD proteolytically processed protein found in inflammatory sites; a 9 amino acid peptide from the C-terminus of chemerin is sufficient to activate ChemR23 (Wittamer *et al.*, 2003, 2004). Chemerin expressed in lymphoid and microvascular endothelium mediates migration of ChemR23-expressing dendritic cells to lymphoid organs and vasculature at sites of inflammation (Vermi *et al.*, 2005). In addition, a bioactive lipid, resolvin E1, was found to functionally interact with ChemR23 to reduce inflammation (Arita et al., 2005). Cloned human ChemR23-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant ChemR23 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at ChemR23.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

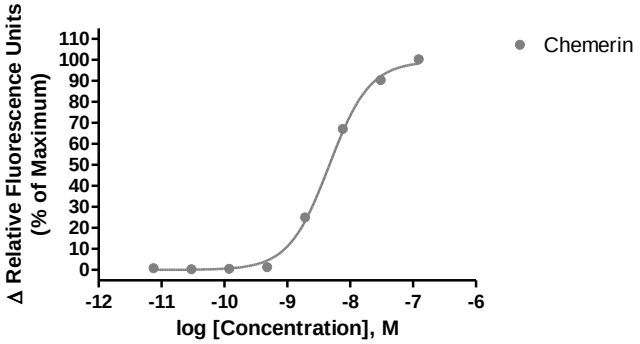

Figure 1. Representative data for activation of ChemR23 receptor. Calcium flux in ChemR23 –expressing Chem-1 cell line induced by Chemerin. ChemR23 –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,400 RLU (Relative Light Units).

Table 1. EC_{50} value of ChemR23 -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Chemerin	Calcium Flux	5	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Chemerin ligand	R&D Systems: 2324-CM
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for $FLIPR^{TETRA}$® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

CHEMR23 cDNA (Accession Number: NM_004072; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

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CODING SEQUENCE

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                                                ATG GAG GAT GAA GAT TAC AAC ACT
TCC ATC AGT TAC GGT GAT GAA TAC CCT GAT TAT TTA GAC TCC ATT GTG GTT TTG GAG GAC
TTA TCC CCC TTG GAA GCC AGG GTG ACC AGG ATC TTC CTG GTG GTG GTC TAC AGC ATC GTC
TGC TTC CTC GGG ATT CTG GGC AAT GGT CTG GTG ATC ATC ATT GCC ACC TTC AAG ATG AAG
AAG ACA GTG AAC ATG GTC TGG TTC CTC AAC CTG GCA GTG GCA GAT TTC CTG TTC AAC GTC
TTC CTC CCA ATC CAT ATC ACC TAT GCC GCC ATG GAC TAC CAC TGG GTT TTC GGG ACA GCC
ATG TGC AAG ATC AGC AAC TTC CTT CTC ATC CAC AAC ATG TTC ACC AGC GTC TTC CTG CTG
ACC ATC ATC AGC TCT GAC CGC TGC ATC TCT GTG CTC CTC CCT GTC TGG TCC CAG AAC CAC
CGC AGC GTT CGC CTG GCT TAC ATG GCC TGC ATG GTC ATC TGG GTC CTG GCT TTC TTC TTG
AGT TCC CCA TCT CTC GTC TTC CGG GAC ACA GCC AAC CTG CAT GGG AAA ATA TCC TGC TTC
AAC AAC TTC AGC CTG TCC ACA CCT GGG TCT TCC TCG TGG CCC ACT CAC TCC CAA ATG GAC
CCT GTG GGG TAT AGC CGG CAC ATG GTG GTG ACT GTC ACC CGC TTC CTC TGT GGC TTC CTG
GTC CCA GTC CTC ATC ATC ACA GCT TGC TAC CTC ACC ATC GTC TGC AAA CTG CAG CGC AAC
CGC CTG GCC AAG ACC AAG AAG CCC TTC AAG ATT ATT GTG ACC ATC ATC ATT ACC TTC TTC
CTC TGC TGG TGC CCC TAC CAC ACA CTC AAC CTC CTA GAG CTC CAC CAC ACT GCC ATG CCT
GGC TCT GTC TTC AGC CTG GGT TTG CCC CTG GCC ACT GCC CTT GCC ATT GCC AAC AGC TGC
ATG AAC CCC ATT CTG TAT GTT TTC ATG GGT CAG GAC TTC AAG AAG TTC AAG GTG GCC CTC
TTC TCT CGC CTG GTC AAT GCT CTA AGT GAA GAT ACA GGC CAC TCT TCC TAC CCC AGC CAT
AGA AGC TTT ACC AAG ATG TCA TCA ATG AAT GAG AGG ACT TCT ATG AAT GAG AGG GAG ACC
GGC ATG CTT TGA
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS071M	ChemiScreen™ ChemR23 Chemoattractant receptor membrane prep

REFERENCES

1. Arita M *et al.* (2005) Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* 201: 713-22.
2. Samson M *et al.* (1998) ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* 28: 1689-700.
3. Vermi W *et al.* (2005) Role of ChemR23 in directing the migration of myeloid and plasmacytoid dendritic cells to lymphoid organs and inflamed skin. *J. Exp. Med.* 201:509-15.
4. Wittamer *et al.* (2003) Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *J. Exp. Med.* 198: 977-985.
5. Wittamer *et al.* (2004) The C-terminal nonapeptide of mature chemerin activates the chemerin receptor with low nanomolar potency. *J. Biol. Chem.* 279: 9956-9962.

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